Regulation of ADAMTS13 Expression in Proliferating Human Umbilical Vein Endothelial Cells

Stephen J. Kling^a Cambria A. Judd^a Keith B. Warner^a George M. Rodgers^{a, b}

^aDivision of Hematology, Department of Internal Medicine, and ^bDepartment of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah, USA

Key Words

ADAMTS13 · Thrombosis · Endothelial cells · von Willebrand factor

Abstract

ADAMTS13, the von Willebrand factor-cleaving protease, is constitutively produced by endothelial cells. The aim was to quantify ADAMTS13 production in proliferating and nonproliferating human umbilical vein endothelial cells (HUVECs) and to determine if such production was regulated. HUVECs were plated at 50,000 cells/cm² or 10,000 cells/cm², densities yielding confluence or subconfluence, respectively. Subconfluent HUVEC supernatants and cell lysates contained at least 2-fold more ADAMTS13 antigen than those of confluent HUVECs ($p < 0.05$). Immunohistochemistry supported this increase and indicated no dramatic shifts in subcellular localization of HUVEC ADAMTS13 by HUVEC proliferation. The activity of the ADAMTS13 produced by subconfluent HUVECs was increased about 2-fold also. HUVECs rapidly increased ADAMTS13 mRNA in response to subconfluent conditions. This change in regulation, in turn, leads to increased ADAMTS13 protein production and activity by these proliferating cells.

Copyright © 2009 S. Karger AG, Basel

Introduction

Von Willebrand factor (VWF), a large multimeric glycoprotein found in plasma, platelets and the subendothelial matrix, is synthesized by endothelial cells (ECs) and megakaryocytes. At sites of vascular injury, VWF promotes thrombus formation through platelet adhesion and thrombus growth through subsequent platelet aggregation. As initially synthesized, VWF is uncleaved, consisting of ultralarge multimers capable of highly efficient thrombus formation, perhaps due to a relative abundance of adhesion sites for platelets and subendothelium [1, 2]. While some of this ultralarge VWF (ULVWF) is stored intracellularly within either endothelial Weibel-Palade bodies or platelet α -granules, most of the synthesized ULVWF is secreted by the ECs [3, 4]. To prevent the constitutive thrombus formation that would otherwise occur upon secretion, ULVWF is immediately cleaved by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) into the more modestly sized VWF multimers which routinely circulate in normal plasma [5]. Severe deficiency of ADAMTS13 (such as autoantibodies, thrombotic thrombocytopenic purpura) leads to circulating ULVWF and, thus, aberrant platelet aggregation, thrombus formation and microvascular occlusion [6, 7].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2009 S. Karger AG, Basel
1424–8832/08/365–0233\$24.50/0

Accessible online at:
www.karger.com/pht

Dr. George M. Rodgers
Division of Hematology, University of Utah Health Sciences Center
30 North 1900 East
Salt Lake City, UT 84132 (USA)
Tel. +1 801 585 3229, Fax +1 801 585 3432, E-Mail george.rodgers@hsc.utah.edu

Initially, ADAMTS13 mRNA expression was found primarily in the liver, specifically in the hepatic stellate cells [8, 9]. ADAMTS13 mRNA expression has since been described in a variety of tissues and cell types, including ECs [10–12]. Using reverse transcription polymerase chain reaction (PCR), amplification of ADAMTS13 mRNA from the liver cell line Hep3B following various stimuli, both inflammatory and immunosuppressive, suggested that any observed decrease in ADAMTS13 activity was not due to transcriptional regulation [13]. More recently, however, a study using real-time PCR found inflammatory cytokines which decreased ADAMTS13 activity but not antigen also decreased ADAMTS13 mRNA in both hepatic stellate cells and human umbilical vein endothelial cells (HUVECs) [14]. Decreases in ADAMTS13 activity have previously been described due to chronic inflammation, sepsis and postoperative stress [15–17]. Regulation of ADAMTS13 production by ECs is of particular interest as ECs produce and secrete ULVWF while also locally controlling coagulation at sites of vascular injury. As proliferating ECs are present at sites of vascular injury, the objective of our study was to determine whether endothelial ADAMTS13 expression was altered and/or regulated by the proliferation state of the ECs.

Materials and Methods

Tissue Culture

HUVECs were isolated from human umbilical veins and cultured in medium 199 (M199; Sigma-Aldrich, St. Louis, Mo., USA) supplemented with 20% fetal bovine serum (Invitrogen Corporation, Carlsbad, Calif., USA), penicillin, streptomycin and amphotericin B [18]. Upon reaching confluence, primary cultures of HUVECs were passaged using 0.25% trypsin/ethylenediamine-tetraacetic acid (EDTA; HyClone, Logan, Utah, USA). Second-passage HUVECs were utilized in experiments and plated at 50,000 cells/cm² for confluent cultures or 10,000 cells/cm² for subconfluent cultures.

ADAMTS13 Enzyme-Linked Immunosorbent Assay

At the times indicated, cellular supernatants were collected and cellular lysates were isolated from adherent second-passage HUVECs cultured in 100-mm dishes (BD Biosciences, Bedford, Mass., USA). Supernatants were centrifuged briefly to remove any cells or cellular debris. Cell monolayers were washed with PBS and lysed with 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSE; EMD Chemicals, La Jolla, Calif., USA) in mammalian protein extraction reagent (M-PER; Pierce, Rockford, Ill., USA) for 5 min, followed by a brief vortex mixing and centrifugation to remove insoluble cell debris. All samples were stored at –70°C until analysis.

Utilizing the IMUBIND® ADAMTS13 enzyme-linked immunosorbent assay (ELISA; American Diagnostica, Stamford, Conn., USA), human ADAMTS13 protein was quantitated in both the cellular supernatant and extracts by absorbance readings on a ThermoMax microplate reader at 450 nm (Molecular Devices, Sunnyvale, Calif., USA) and comparison to a standard curve. For normalization purposes, total cellular protein concentrations of the cell lysates were determined using a BCA protein assay (Pierce).

Immunohistochemistry

Adherent second-passage HUVECs cultured in 24-well cell culture plates (BD Biosciences) were rinsed with phosphate-buffered saline (PBS) after 24 h and fixed in 4% paraformaldehyde for 15 min at room temperature. Samples were permeabilized 20 min in 0.2% Triton X-100/PBS, washed 3 times with PBS, and incubated in blocking buffer (5% FBS in PBS) for 1 h before incubation at 4°C overnight in 1 µg/ml mouse monoclonal anti-human ADAMTS13 (Abcam Inc., Cambridge, Mass., USA) in blocking buffer. Samples were then washed 3 times with PBS and incubated in the dark for 2 h at 4°C in 10 µg/ml Cy3-conjugated goat anti-mouse IgG (KPL Inc., Gaithersburg, Md., USA) in blocking buffer. Samples were again washed 3 times with PBS, and images acquired at 20× with an Olympus DP71 charge-coupled device (CCD) camera mounted on an Olympus IX70 microscope using Cy3 filters and DP Manager software (version 3.1.1.208). Fluorescence intensity per cell was quantitated using ImageJ software (version 1.42a). For cell count experiments, parallel cultures of confluent and subconfluent HUVECs were grown 24, 48, 72 or 96 h. These cultures were either lysed for total cellular lysate isolation, as described above, or fixed and stained by washing with PBS, fixing for 10 min in 4% paraformaldehyde, and staining for 15 min with 1 µg/ml DAPI (KPL Inc.). Images for these cell count experiments were again acquired using an Olympus DP71 CCD camera mounted on an Olympus IX70 microscope (10× with DAPI filters). Nuclei were counted using ImageJ software. Routine assessment of the endothelial phenotype was performed by staining for VWF (goat anti-human VWF from Haematologic Technologies Inc., Essex Junction, Vt., USA; rabbit anti-goat IgG-HRP from Pierce; DAB from Sigma).

ADAMTS13 Activity

At the times indicated following passage, supernatants were collected from adherent second-passage HUVECs cultured in 100-mm dishes and the cell layers washed with PBS and lysed with 1 mM AEBSE in M-PER for 5 min, followed by a brief vortex mixing and centrifugation to remove insoluble cell debris. Samples were stored at –70°C until analysis.

Utilizing a commercially available fluorescence resonance energy transfer-based assay, ACTIFLUOR™ ADAMTS13 Activity Assay (American Diagnostica), ADAMTS13 activity was quantitated in cellular extracts and supernatants by fluorescence readings on a SpectraMax M5 microplate reader at 485 nm excitation/535 nm emission/530 nm cutoff (Molecular Devices, Sunnyvale, Calif., USA) and comparison to a standard curve [19]. For normalization purposes, total protein concentrations of the cell lysates were determined using a BCA protein assay. The presence of serum in the culture medium precluded any meaningful quantitation of total cellular protein within cellular supernatants.

Real-Time PCR

At the times indicated following passage, total RNA from cell layers grown in 100-mm dishes was isolated using the RNeasy Plus mini kit (QIAGEN; Valencia, Calif., USA). At time points earlier than one hour, insufficient HUVECs had adhered to form an adherent monolayer, and thus, RNA isolation was not performed before one hour. 0.5 µg of purified total RNA from each sample was reverse-transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, Calif., USA). The mRNA was quantified by real-time PCR on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems) using commercially available primer probe sets for ADAMTS13 and GAPDH mRNA (TaqMan gene expression assays; Applied Biosystems). The relative abundance of ADAMTS13 mRNA transcripts was normalized to GAPDH, and the normalized ADAMTS13 mRNA from the earliest confluent culture (i.e., 1 h confluent mRNA) was set as 100%. To estimate mRNA half-life, 5 µg/ml actinomycin D (Sigma-Aldrich) was added to cultures 2 h after passage, mRNA isolated at various times following the addition of actinomycin D, and amplification performed as described above.

Statistics

Data are expressed as means $\pm$ standard deviations of triplicate cultures. ELISA and activity assay data are normalized per microgram of total cellular protein. Real-time PCR data are normalized to GAPDH mRNA expression, which appeared comparable at the different cell densities used. All data are representative of at least three separate experiments performed. Statistical significance ($p < 0.05$) was determined using unpaired Student's *t* test.

Results

ADAMTS13 Enzyme-Linked Immunosorbent Assay

To determine whether the growth state of HUVECs affects ADAMTS13 protein expression, the accumulated ADAMTS13 protein secreted into the supernatant from time zero (that is, plating) to the indicated time was quantitated by ELISA, and the results normalized to total cellular protein (fig. 1a). 24 h after passage, the supernatants from subconfluent HUVEC cells contained about 6 pg/ml of ADAMTS13 compared to 2 pg/ml from those of confluent HUVECs, a 3-fold increase in ADAMTS13. 48 h after passage, however, the supernatants from subconfluent HUVECs contained 2-fold more ADAMTS13 than those of confluent HUVECs (4 vs. 2 pg/ml). This 2-fold increase was sustained at the 72 and 96 h time points.

Intracellular ADAMTS13 protein contained within cellular lysates from time zero (that is, plating) to the indicated time was quantitated by ELISA and the results normalized to total cellular protein (fig. 1b). 24 h after passage, the lysates from subconfluent HUVECs con-

tained about 60 pg/ml of ADAMTS13 compared to 16 pg/ml from those of confluent HUVECs, a nearly 4-fold increase in ADAMTS13. 48 h after passage, however, the lysates from subconfluent HUVECs contained nearly 2-fold more ADAMTS13 than those of confluent HUVECs (25.5 vs. 15.9 pg/ml). This approximately 2-fold increase was sustained at the 72 and 96 h time points.

Total cellular protein from the HUVEC cellular lysates was quantitated by a BCA assay for normalization purposes (fig. 1c). While the total protein amounts ranged from about 530 to 590 µg per 100-mm dish of confluent HUVECs, total protein amounts gradually increased over time from about 120 µg at 24 h to over 400 µg at 96 h per 100-mm dish of subconfluent HUVECs. To test whether total cellular protein directly correlated with cell number regardless of proliferative state, parallel HUVEC cultures were either lysed, to quantitate total cellular protein, or fixed and stained, to count nuclei and thus cell number (fig. 1d). The amount of protein per cell remained constant in both the quiescent confluent HUVECs and the actively proliferating subconfluent HUVECs, with no significant differences observed between the groups.

Immunohistochemistry

To verify the observed increase in ADAMTS13 protein expression, confluent and subconfluent HUVECs were fluorescently stained for ADAMTS13 expression. 24 h following passage, subconfluent HUVECs demonstrated more intense intracellular staining compared with confluent cultures (fig. 2a). The fluorescence intensity of subconfluent HUVECs was about 89,992 compared to 33,801 for confluent HUVECs, a nearly 3-fold increase in ADAMTS13 (fig. 2b). While quantitatively different, the actual subcompartmental location within the subconfluent HUVECs appeared primarily perinuclear and no different from that of confluent HUVECs.

ADAMTS13 Activity

To determine whether the ADAMTS13 protein detected was capable of cleaving VWF, a fluorescence resonance energy transfer-based activity assay was used to quantitate ADAMTS13 activity. ADAMTS13 activity in cell supernatants from time zero (that is, plating) to the indicated time was quantitated and the results normalized to total cellular protein (fig. 3a). 24 h after passage, the activity in supernatants from subconfluent HUVECs was equivalent to about 29 pg/ml compared to 9 pg/ml from those of confluent HUVECs, a 3-fold increase in ADAMTS13 activity. 48 h after passage, however, the activity in supernatants from subconfluent HUVECs was

Fig. 1. Intracellular and secreted ADAMTS13 is increased in subconfluent HUVECs. Second-passage HUVECs were plated at 50,000/cm² (confluent) or 10,000/cm² (subconfluent). At the indicated times after passage, cellular supernatants (**a**) were collected and cellular lysates (**b**) extracted. ADAMTS13 antigen was quantitated using the IMUBIND ADAMTS13 ELISA kit. Total cellular protein from lysates was determined using a BCA protein assay (**c**). Cell counts were determined by fixation and DAPI staining parallel cultures, acquiring fluorescent images using an

Olympus DP71 CCD camera mounted on an Olympus IX70 microscope, and counting nuclei using ImageJ (version 1.42a). Normalization of total cellular protein to cell number appears in **d**. Data are shown as means $\pm$ standard deviations of triplicate cultures, normalized per microgram of total cellular protein, and are representative of three experiments performed. Statistical significance ($p < 0.05$) between confluent and subconfluent at each time point is indicated by asterisks.

only 2-fold greater than that of confluent HUVECs (22 vs. 12 pg/ml). The increased ADAMTS13 activity observed in supernatants from subconfluent HUVECs continued to decline at the later time points, with only a 50% increase observed after 96 h. Addition of EDTA (20 mM final concentration) to the samples ablates the observed ADAMTS13 activity (data not shown).

The ADAMTS13 activity contained within cellular lysates from time zero (that is, plating) to the indicated time was also quantitated, and the results normalized to total cellular protein (fig. 3b). 24 h after passage, the activity in lysates from subconfluent HUVECs was equiv-

alent to about 150 pg/ml of ADAMTS13 compared to 70 pg/ml from those of confluent HUVECs, a 2-fold increase in ADAMTS13 activity. 48 h after passage, however, the activity in lysates from subconfluent HUVECs was only about 50% greater than that of confluent HUVECs (122 vs. 78 pg/ml). This 50% increase in ADAMTS13 activity observed in lysates from subconfluent HUVECs was sustained at the 72 and 96 h time points.

Real-Time PCR

To determine whether the increased ADAMTS13 protein detected was due to transcriptional regulation, the

Fig. 2. Intracellular ADAMTS13 is increased in subconfluent HUVECs. Second-passage HUVECs were plated at 50,000/cm² (confluent) or 10,000/cm² (subconfluent). After 24 h, the adherent HUVEC cell layers were washed, fixed with 2% formaldehyde, blocked and stained with mouse monoclonal anti-human ADAMTS13 primary antibody, followed by a Cy3-conjugated goat anti-mouse IgG secondary antibody. Images (20×) were acquired using an Olympus DP71 CCD camera mounted on an Olympus IX70 microscope (**a**). Fluorescence intensity per cell (**b**) was determined by analyzing images with ImageJ (version 1.42a) and is depicted as means ± standard deviations. Data are representative of quadruplicate cultures from more than four experiments performed. Statistical significance ($p < 0.05$) between confluent and subconfluent is indicated by asterisks.

relative abundance of ADAMTS13 mRNA was quantitated by real-time PCR (fig. 4). Within 2 h of passage, subconfluent HUVECs contained 2-fold more ADAMTS13 mRNA than confluent HUVECs. By 6 h, the increased ADAMTS13 mRNA expression by subconfluent HUVECs was only 30% more than confluent

Fig. 3. HUVEC ADAMTS13 is functional and increased in subconfluent HUVECs. Second-passage HUVECs were plated at 50,000/cm² (confluent) or 10,000/cm² (subconfluent). At the times indicated after passage, cellular supernatants were collected and cellular lysates were extracted. Total cellular protein from lysates was determined using a BCA protein assay. ADAMTS13 activity in cellular supernatants (**a**) and in cellular lysates (**b**) was determined using the ACTIFLUOR ADAMTS13 Activity kit. Data are shown as means ± standard deviations of triplicate cultures, normalized per microgram of total cellular protein, and are representative of two experiments performed. Statistical significance ($p < 0.05$) between confluent and subconfluent at each time point is indicated by asterisks.

HUVECs, and by 12 h, ADAMTS13 mRNA expression was not significantly different. In mRNA stability studies with actinomycin D, the ADAMTS13 half-life was not significantly different between confluent and subconfluent HUVEC cultures (8.6 ± 0.4 vs. 8.2 ± 0.1 h, $p < 0.246$; data not shown).

Fig. 4. ADAMTS13 mRNA is increased in subconfluent HUVECs. Second-passage HUVECs were plated at 50,000/cm² (confluent) or 10,000/cm² (subconfluent). At the indicated times following plating, total mRNA was isolated from the adherent HUVECs. 0.5 µg of total mRNA was reverse transcribed and then amplified using real-time PCR. All samples were compared to the normalized ADAMTS13 mRNA from the earliest confluent culture (that is, 1 h confluent mRNA), which was set to 100%. Data are shown as means ± standard deviations of triplicate cultures, normalized to GAPDH expression per sample, and are representative of three separate experiments performed. Statistical significance ($p < 0.05$) between confluent and subconfluent at each time point is indicated by asterisks.

Discussion

In order to maintain homeostasis, the vascular endothelium, which acts as a barrier between underlying somatic cells and the circulating blood, affects angiogenesis, vasodilation and vasoconstriction, permeability, response to inflammation, and hemostasis. In the quiescent, unperturbed state, confluent ECs inhibit coagulation and platelet function to allow blood to freely circulate. Activation or injury of ECs, on the other hand, leads to the release of intracellular ULVWF [1], which is immediately cleaved by ADAMTS13. The resulting VWF is free to bind to the basement membrane and platelets, initiating thrombus formation. Although earlier studies have shown that ECs, including HUVECs, constitutively express ADAMTS13, this is the first study to demonstrate increased transcriptional regulation and expression of ADAMTS13 in HUVECs.

As ADAMTS13 is constitutively expressed, the consistent amount of ADAMTS13 protein expressed by confluent HUVECs over time may merely reflect the quiescent

state of these cells producing a constitutive protein. The increased ADAMTS13 expression observed in subconfluent HUVECs, on the other hand, may reflect their proliferative state. Proliferating cells, being more metabolically active, are assumed to produce more protein than quiescent nondividing cells. The observed constant HUVEC total cellular protein content per cell, regardless of proliferative status, however, implies that, if indeed proliferating HUVECs produce more of some proteins, the production of other proteins must necessarily be downregulated in order to not significantly alter total cellular protein levels.

The 3- to 4-fold initial increase in ADAMTS13 protein expression by subconfluent HUVECs observed at 24 h decreased to a steady-state level of about 2-fold at subsequent time points. This increased expression differs from the consistent expression by confluent HUVECs. Whether this difference results from the response of quiescent versus proliferating cells to perturbation by cell dissociation or from cells staying in or leaving contact inhibition cannot be discerned. In either case, the subconfluent HUVECs' response differs from confluent HUVECs' by this stimulus. The switch to a proliferative state might account for the initial 3- to 4-fold elevation of ADAMTS13 observed in subconfluent HUVECs. With the relatively long ADAMTS13 half-life of 2–3 days [20], significant alterations in ADAMTS13 protein could persist for days even if the change were transitory, diminishing as the cells approach confluence.

Immunohistochemical staining supported the increased intracellular ADAMTS13 observed in subconfluent HUVECs. Also, there did not appear to be any dramatic shifts in intracellular subcompartmental localization of ADAMTS13. Others have noted that ADAMTS13 colocalizes with VWF in the endoplasmic reticulum, Golgi apparatus and some secretory granules, but not Weibel-Palade bodies [21]. As such, the increased potential for intracellular cleavage of ULVWF [22] with increased intracellular ADAMTS13 could prepare subconfluent HUVECs for regulation of thrombus formation and growth at the very sites where they are likely to be – sites of vascular injury.

ADAMTS13 activity was consistently greater in lysates and supernatants from subconfluent HUVECs than from confluent HUVECs. These results coincided with the antigen/protein results. While overall activity levels were greater in subconfluent cells throughout the experiments, a gradual decline in ADAMTS13 activity was observed at later time points in the subconfluent HUVEC lysates and supernatants. This decrease might result from

increases in ADAMTS13's substrate, ULVWF. Increases in ULVWF, as stimulated by inflammatory cytokines, for example, can inhibit ADAMTS13 activity [23]. Since the functional ability of ADAMTS13 to physically cleave ULVWF is not affected by removal of the propeptide region of ADAMTS13 but by the folding of the protein itself [24], excess ULVWF within even shared subcompartments (that is, endoplasmic reticulum, Golgi apparatus and certain secretory granules) could negatively impact ADAMTS13 activity levels.

Increased intracellular and secreted ADAMTS13 protein expression by subconfluent HUVECs suggests either an increase in production via transcription and translation or a decrease in some regulator or protease via protein degradation. A variety of plasma proteases have in fact been described which degrade ADAMTS13, including thrombin, factor Xa and plasmin [24]. Increased ADAMTS13 mRNA expression supports transcriptional upregulation as the source of increased ADAMTS13 in subconfluent HUVECs. While early studies suggested transcriptional regulation of ADAMTS13 seemed unlikely [13], a recent report found inflammatory cytokines downregulated ADAMTS13 mRNA in both hepatic stellate cells and ECs [14]. Again, with the relatively long ADAMTS13 protein half-life of 2–3 days [25], significant but transient alterations in ADAMTS13 mRNA could result in ADAMTS13 protein levels which persist for days, allowing time for the cells to reach confluence and steady-state levels of ADAMTS13 production.

ECs synthesize and secrete ULVWF, which is quickly cleaved on or near the EC surface into the less thrombogenic circulating VWF. ECs also synthesize and secrete ADAMTS13, which is responsible for the quick cleavage of ULVWF into VWF. At sites of vascular injury, VWF attaches to the exposed basement membrane and tethers circulating platelets, allowing for the further aggregation of platelets and formation of the primary platelet plug. Proliferating ECs, which re-endothelialize sites of vascular injury and can form new blood vessels, are likely found at sites with higher VWF protein concentrations, for example thrombi and plaques [26, 27].

Although hepatic stellate cells are known to be the primary source of circulating ADAMTS13 [9], the ability of proliferating ECs to regulate their expression of ADAMTS13 mRNA leading to increased local ADAMTS13 antigen and activity may be important in further modulating VWF-mediated thrombus formation and growth at specific sites of vascular injury, thus aiding in vessel wall repair via the reestablishment of the vessel's underlying cells and ultimately the luminal endothelial

barrier. Posttranslational regulation of ADAMTS13 by proteolysis (such as thrombin or factor Xa) could further modulate thrombus growth locally, as these enzymes, already present at sites of thrombosis, allow for a reduction in ADAMTS13 activity and thereby an increase in thrombus growth [24].

Proliferating HUVECs are known to synthesize more VWF [28] and, with this study, are now known to synthesize more ADAMTS13. As damaged vessels are repaired, the presence of proliferating ECs, along with their increased ADAMTS13, would allow for micromanagement of thrombus formation/growth, particularly in microvessels where high shear stress increases the thrombogenic effect of ULVWF. Increased ADAMTS13 by proliferating ECs would allow for decreasing thrombus size, aiding recolonization by smooth muscle cells, fibroblasts and ECs to repair the vessel wall, and increasing the potential that, if these proliferating ECs were injured, the released elevated ADAMTS13 would cleave the elevated ULVWF, which would also be released by injured proliferating ECs. Thus, the ability to upregulate ADAMTS13 may prove useful not only in treating ADAMTS13 deficiencies, such as thrombotic thrombocytopenic purpura, but also inflammation, surgery, stress or other trauma, all of which result in decreased ADAMTS13.

Acknowledgments

The authors wish to thank: Hugh L. Fryer (American Diagnostica) who supplied ADAMTS13 antigen and activity assay kits; Chris Rodesch and the imaging facility at the University of Utah Cell Imaging Core; Michael Klein and the real-time PCR facility at the University of Utah Genomics Core. Funding for these studies was provided by the ARUP Institute of Clinical and Experimental Pathology.

References

- 1 Wagner DD: Cell biology of von Willebrand factor. *Annu Rev Cell Biol* 1990;6:217–246.
- 2 Ruggeri ZM: Von Willebrand factor, platelets and endothelial cell interactions. *J Thromb Haemost* 2003;1:1335–1342.
- 3 Bowie EJ, Solberg LA Jr, Fass DN, Johnson CM, Knutson GJ, Stewart ML, Zoccklein LJ: Transplantation of normal bone marrow into a pig with severe von Willebrand's disease. *J Clin Invest* 1986;78:26–30.
- 4 Tsai HM, Nagel RL, Hatcher VB, Seaton AC, Sussman II: The high molecular weight form of endothelial cell von Willebrand factor is released by the regulated pathway. *Br J Haematol* 1991;79:239–245.

- 5 Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, Schade AJ, McIntire LV, Fujikawa K, Lopez JA: ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 2002;100:4033–4039.
- 6 Furlan M, Robles R, Solenthaler M, Lämmle B: Acquired deficiency of von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood* 1998;91:2839–2846.
- 7 Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, Krause M, Scharer I, Aumann V, Mittler U, Solenthaler M, Lämmle B: von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med* 1998;339:1578–1584.
- 8 Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, Yang AY, Siemieniak DR, Stark KR, Gruppo R, Sarode R, Shurin SB, Chandrasekaran V, Stabler SP, Sabio H, Bouhassira EE, Upshaw JD Jr, Ginsburg D, Tsai HM: Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001;413:488–494.
- 9 Zhou W, Inada M, Lee TP, Bente D, Lyubsky S, Bouhassira EE, Gupta S, Tsai HM: ADAMTS13 is expressed in hepatic stellate cells. *Lab Invest* 2005;85:780–788.
- 10 Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K: Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem* 2001;276:41059–41063.
- 11 Plaimauer B, Zimmermann K, Völkel D, Antoine G, Kerschbaumer R, Jenab P, Furlan M, Gerritsen H, Lämmle B, Schwarz HP, Scheiflinger F: Cloning, expression, and functional characterization of the von Willebrand factor-cleaving protease (ADAMTS13). *Blood* 2002;100:3626–3632.
- 12 Turner N, Nolasco L, Tao Z, Dong JF, Moake J: Human endothelial cells synthesize and release ADAMTS-13. *J Thromb Haemost* 2006;4:1396–1404.
- 13 Claus RA, Bockmeyer CL, Kentouche K, Sieber MW, Oberle V, Kaufmann R, Deigner HP, Lösche W: Transcriptional regulation of ADAMTS13. *Thromb Haemost* 2005;94:41–45.
- 14 Cao WJ, Niiya M, Zheng XW, Shang DZ, Zheng XL: Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells. *J Thromb Haemost* 2008;6:1233–1235.
- 15 Bockmeyer CL, Claus RA, Budde U, Kentouche K, Schneppenheim R, Lösche W, Reinhart K, Brunkhorst FM: Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor. *Haematologica* 2008;93:137–140.
- 16 Kremer Hovinga JA, Zeerleder S, Kessler P, Romani de Wit T, van Mourik JA, Hack CE, ten Cate H, Reitsma PH, Willemin WA, Lämmle B: ADAMTS-13, von Willebrand factor and related parameters in severe sepsis and septic shock. *J Thromb Haemost* 2007;5:2284–2290.
- 17 Mannucci PM, Parolari A, Canciani MT, Alemanni F, Camera M: Opposite changes of ADAMTS-13 and von Willebrand factor after cardiac surgery. *J Thromb Haemost* 2005;3:397–399.
- 18 Jaffe EA, Nachman RL, Becker CG, Minick CR: Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* 1973;52:2745–2756.
- 19 Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T: FRET-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol* 2005;129:93–100.
- 20 Furlan M, Robles R, Morselli B, Sandoz P, Lämmle B: Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in patients with thrombotic thrombocytopenic purpura. *Thromb Haemost* 1999;81:8–13.
- 21 Shang D, Zheng XW, Niiya M, Zheng XL: Apical sorting of ADAMTS13 in vascular endothelial cells and Madin-Darby canine kidney cells depends on the CUB domains and their association with lipid rafts. *Blood* 2006;108:2207–2215.
- 22 Majerus EM, Zheng X, Tuley EA, Sadler JE: Cleavage of the ADAMTS13 propeptide is not required for protease activity. *J Biol Chem* 2003;278:46643–46648.
- 23 Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF: Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood* 2004;104:100–106.
- 24 Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS, Lane DA: Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. *Blood* 2005;105:1085–1093.
- 25 Furlan M, Robles R, Morselli B, Sandoz P, Lämmle B: Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in patients with thrombotic thrombocytopenic purpura. *Thromb Haemost* 1999;81:8–13.
- 26 Yamashita A, Asada Y, Sugimura H, Yamamoto H, Marutsuka K, Hatakeyama K, Tamura S, Ikeda Y, Sumiyoshi A: Contribution of von Willebrand factor to thrombus formation on neointima of rabbit stenotic iliac artery under high blood-flow velocity. *Arterioscler Thromb Vasc Biol* 2003;23:1105–1110.
- 27 De Meyer GR, Hoylaerts MF, Kockx MM, Yamamoto H, Herman AG, Bult H: Intimal deposition of functional von Willebrand factor in atherosclerosis. *Arterioscler Thromb Vasc Biol* 1999;19:2524–2534.
- 28 Blann AD: Increased release of von Willebrand factor antigen by endothelial cells whilst in active growth phase. *Cell Biol Int Rep* 1991;15:493–498.